Macrophages: versatile players in renal inflammation and fibrosis

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Abstract | Macrophages have important roles in immune surveillance and in the maintenance of kidney homeostasis; their response to renal injury varies enormously depending on the nature and duration of the insult. Macrophages can adopt a variety of phenotypes: at one extreme, M1 pro-inflammatory cells contribute to infection clearance but can also promote renal injury; at the other extreme, M2 anti-inflammatory cells have a reparative phenotype and can contribute to the resolution phase of the response to injury. In addition, bone marrow monocytes can differentiate into myeloid-derived suppressor cells that can regulate T cell immunity in the kidney. However, macrophages can also promote renal fibrosis, a major driver of progression to end-stage renal disease, and the $CD206^+$ subset of M2 macrophages is strongly associated with renal fibrosis in both human and experimental diseases. Myofibroblasts are important contributors to renal fibrosis and recent studies provide evidence that macrophages recruited from the bone marrow can transition directly into myofibroblasts within the injured kidney. This process is termed macrophage-to-myofibroblast transition (MMT) and is driven by transforming growth factor-β1 (TGFβ1)–Smad3 signalling via a Src-centric regulatory network. MMT may serve as a key checkpoint for the progression of chronic inflammation into pathogenic fibrosis.

Macrophages are heterogeneous cells that form an important part of the phagocytic system[1,2], and they were first recognized for their ability to clear infectious microorganisms[3]. Subsequent studies revealed that macrophages are multifunctional cells that have distinct biological functions depending on the local microenvironment[4–8]; they primarily have a sentinel role in pathogen clearance and in the repair of injured tissues[9–11] but can also promote tissue damage. Macrophages can be found in a variety of tissues, and under healthy physiological conditions the kidney macrophage compartment includes a population of long-lived tissue-resident macrophages as well as macrophages that have differentiated from circulating monocytes that are produced in the bone marrow[12,13].

A pathogenic role for macrophages has been proposed in many types of human disease, including kidney disease[14–19]. This proposal is supported by studies of animal models that have defined the contribution of macrophages and their products to kidney injury and repair[20–26]. Some of the latest developments in this area of research have elucidated the role of macrophage plasticity during the progression from kidney inflammation to renal fibrosis — an important step in the development of end-stage renal disease (ESRD)[17,27–29]. In this Review, we discuss the varied roles of macrophages in renal pathology and consider therapeutic avenues to target macrophage-dependent kidney damage.

Macrophage heterogeneity and plasticity

Macrophages can adopt many distinct phenotypes under normal physiological conditions and, even more so, in the context of tissue damage when the local microenvironment is disrupted by factors such as pathogens, cellular damage, innate and adaptive immune responses, hypoxia and tissue repair processes. Following recruitment into damaged tissue, monocytes can differentiate into a variety of macrophage phenotypes (FIG. 1). The differentiation programmes involved in the generation of different macrophage phenotypes have been categorized as classical M1-type activation and alternative M2-type activation in an effort to align them with the paradigm of T cell differentiation into T helper 1 (T_H1) and T_H2 cells. These T_H cells have distinct functional properties and specialize in the elimination of different types of pathogens — usually intracellular microorganisms such as viruses in the case of T_H1 cells and extracellular parasites such as helminths in the case of T_H2 cells[30,31]. The categorization of M1 and M2 macrophages stems from analyses of gene expression patterns and the functional potential of cultured macrophages following their exposure to various stimuli. However, although the terms M1

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Key points

- Macrophages have important roles in kidney homeostasis and in the response to acute and chronic kidney injury.
- Macrophage phenotype varies enormously depending on the nature and duration of renal injury, ranging from the pro-inflammatory phenotype of M1 cells to the anti-inflammatory phenotype of M2 cells that are involved in tissue repair and fibrosis; monocytic myeloid-derived suppressor cells contribute to immune regulation.
- Macrophages are plastic cells and their gene expression patterns and functions adapt rapidly to the dynamics of the renal microenvironment.
- $CD206^+$ M2 macrophages are strongly associated with renal fibrosis in human and experimental kidney diseases.
- Macrophages derived from bone marrow cells can directly contribute to renal fibrosis through a process termed macrophage-to-myofibroblast transition (MMT).
- The induction of MMT, via the Src-centric regulatory network mediated by transforming growth factor-β1 (TGFβ1)–Smad3, serves as a key checkpoint in the progression of chronic inflammation to renal fibrosis.

and M2 are widely used to describe macrophage populations in studies of human and experimental disease, and indeed, we use the terms M1 and M2 to describe macrophages in this Review, it is important to note that the paradigm of M1 and M2 macrophages represents a major oversimplification of macrophage phenotype and function. In fact, a continuum of macrophage polarization states exists, which can be appreciated by analysing macrophage populations at the single-cell level. Indeed, single-cell RNA-sequencing (scRNA-seq) has revealed the immense complexity of macrophage populations in the development and progression of diseases such as cancer and atherosclerosis[32–34]. It is likely that the insights gained from such analyses at the single-cell level will lead to new approaches for categorizing macrophages according to their phenotype and function. Resident kidney macrophages, for example, represent a distinct macrophage subset that can promote angiogenesis and repair in the damaged kidney[35].

In the presence of pathogenic organisms such as yeast and bacteria, monocytes can adopt an M1 pro-inflammatory phenotype[36,37]. M1 macrophages have an important role in host defence and promote a T_H1-like immune response[30] (FIG. 1). M1 polarization can be triggered by pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS), danger-associated molecular patterns (DAMPs; also known as alarmins) such as S100A9 and IL-1α and pro-inflammatory cytokines such as IFNγ and tumour necrosis factor (TNF)[38–41]. This M1 polarization is characterized by the upregulation of cell surface activation markers and molecules involved in antigen presentation, such as major histocompatibility complex (MHC) class II, CD16, CD32, CD80, CD86 and IL-1 receptor (IL-1R), as well as the production of a range of pro-inflammatory molecules, such as IL-1, IL-6, IL-12, IL-23, inducible nitric oxide synthase (NOS2; also known as iNOS), matrix metalloproteinase 12 (MMP12) and macrophage-inducible C-type lectin (MINCLE)[42–47].

Monocytes that infiltrate damaged tissue can also undergo alternative activation to adopt an M2 phenotype[30] (FIG. 1). M2 macrophages have a wide range of functions, which include the suppression of inflammation and promotion of tissue repair[48–51], and can be classified into three M2 subpopulations (that is, M2a–M2c) on the basis of their phenotype and function. Macrophage polarization into M2 phenotypes is driven by a diverse range of stimuli: IL-4 and IL-13 induce the differentiation of M2a macrophages[52,53], immune complexes and Toll-like receptor (TLR) and/or IL-1R ligands induce the differentiation of M2b macrophages[54,55], and IL-10, transforming growth factor-β (TGFβ) and glucocorticoids induce the differentiation of M2c macrophages[56]. The subtypes of M2 macrophages have distinct and overlapping functions: M2a macrophages induce an anti-inflammatory T_H2-like immune response and promote wound healing and tissue fibrosis; M2b macrophages participate in immunoregulation and contribute to T_H2-like activation; and M2c macrophages contribute to immunosuppression, matrix remodelling and tissue repair[57–60]. However, studies of M2 macrophages in the injured kidney are hampered by a lack of cell surface markers that can distinguish between these diverse functional populations.

Macrophage plasticity

Monocytes and macrophages can be polarized to adopt a particular phenotype in response to specific stimuli, but whether the same cell can then undergo a further phenotypic change secondary to changes in the microenvironment is unclear. This possibility is particularly relevant in conditions in which a clear M1-to-M2 phenotypic switch occurs, such as in renal ischaemia–reperfusion injury (IRI), whereby the early infiltrate of macrophages predominantly has an M1 pro-inflammatory phenotype but the infiltrate present during the tissue repair phase has a predominantly M2 phenotype[49,50,61]. Whether the early M1 macrophages switch phenotype to become M2 macrophages or whether these are separate cell populations remains unclear. This question was addressed in a study whereby different macrophage populations were polarized into an M2 phenotype in vitro before being adoptively transferred into recipient mice with adriamycin-induced nephropathy. Notably, polarized M2 splenic macrophages maintained their phenotype in the damaged kidney and protected against adriamycin-induced nephropathy, whereas polarized M2 bone marrow macrophages underwent a proliferation-dependent phenotype switch and failed to provide protection[62], showing that bone-marrow-derived macrophages have considerable plasticity.

Monocyte-derived macrophage diversity

Considerable heterogeneity exists in the monocyte and macrophage populations that originate from bone marrow cells. This heterogeneity is best illustrated by the distinct populations of mouse blood monocytes that are defined according to their levels of expression of the cell surface marker Ly6C[63]. Most kidney-infiltrating macrophages observed in models of acute kidney injury (AKI) are derived from the circulating $Ly6C^{high}$ monocyte population[64]. Infiltrating $Ly6C^{high}$ macrophages within the kidney appear morphologically immature and monocyte-like, whereas the $Ly6C^{low}$ population, which is derived from infiltrating $Ly6C^{high}$ monocytes,

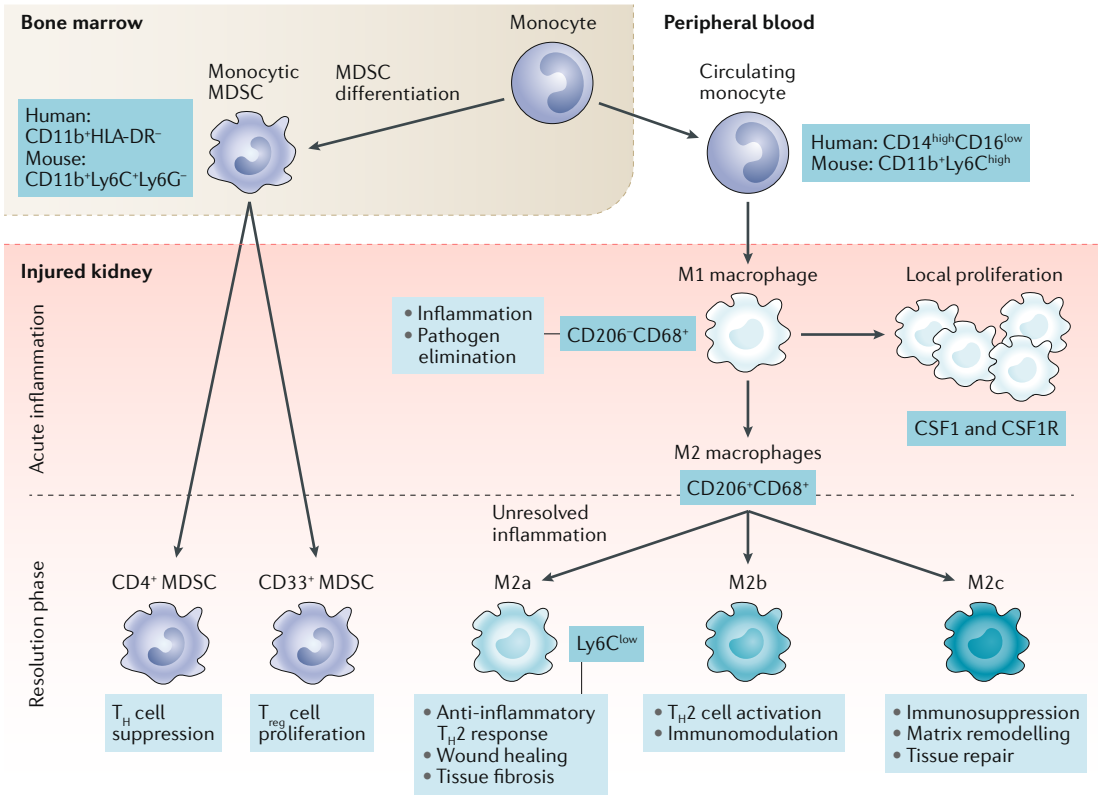

Fig. 1 | **Phenotype and function of bone-marrow-derived monocytes and macrophages in the injured kidney.** In response to kidney injury, the bone marrow increases the production and release of monocytes into the peripheral blood. Circulating classical monocytes ($CD11b^+Ly6C^{high}$ in the mouse or $CD14^{high}CD16^{low}$ in humans) are recruited to the kidney, where they differentiate into pro-inflammatory M1 macrophages during the early phase of renal injury, in response to infection or cell damage[45,127,259]. This differentiation is followed by a change in the macrophage infiltrate, as the cells predominantly adopt an anti-inflammatory M2 phenotype throughout the later stages of tissue repair[42,48,50,210]. During the tissue repair phase, M2b and M2c macrophages are mainly responsible for immunosuppression, matrix remodelling and wound healing once the underlying injury has resolved[9,52,57,59,60,182,260]. Monocytic myeloid-derived suppressor cells (MDSCs) produced in the bone marrow can also be recruited into the injured kidney, where they can suppress effector T cell activation and promote regulatory T (T_{reg}) cell proliferation[69]. By contrast, unresolved renal inflammation triggers M2a macrophage polarization in the injured kidney via IL-4 and IL-13 (REF.[52]), which results in increased transforming growth factor-β1 (TGFβ1) production and renal fibrosis[261,262]. CSF1, colony-stimulating factor 1; CSF1R, CSF1 receptor; T_H, T helper cell.

is more mature and transcribes genes consistent with a selective profibrotic function[64,65]. The numbers of $Ly6C^{int}$ macrophages in the injured kidney peak during kidney repair, and these cells have a distinct wound healing phenotype; this phenotype was confirmed by inhibiting macrophage colony-stimulating factor 1 receptor (CSF1R) to suppress macrophage proliferation at different disease stages in a renal IRI model[66]. There is also a $Ly6C^-$ population of circulating blood monocytes that is characterized by prolonged crawling along the endothelium; these cells phagocytose microparticles and soluble material from the bloodstream and can respond to microbial infection and tissue injury[67]. These patrolling monocytes have also been identified within the kidney, where they present antigen and activate $CD4^+$ T cells within glomerular capillaries[68].

A subset of cells from the monocyte lineage in bone marrow gives rise to monocytic myeloid-derived suppressor cells (MDSCs), which have a suppressive effect on the immune response[69]. Monocytic MDSCs induce tolerance in a mouse model of cardiac allograft rejection, whereas in vitro-generated monocytic MDSCs can suppress rejection of male-to-female skin grafts through a NOS2-dependent mechanism[70,71].

It is important to note that macrophage biology clearly overlaps with that of dendritic cells in terms of their cellular origin and morphology, their function as phagocytic and antigen-presenting cells, and their cell surface antigen expression; for this reason, macrophages and dendritic cells in the kidney are sometimes broadly referred to as kidney mononuclear phagocytes[72]. The role of dendritic cells in kidney disease, however, is beyond the scope of this Review and has been reviewed elsewhere[1,73].

Acute kidney injury

Macrophage accumulation in the glomerular and tubulointerstitial compartments is a prominent feature of acute and chronic inflammatory kidney disease. This accumulation results from the recruitment of blood

Clodronate liposomes
Phospholipid bilayers (that is, liposomes) that encapsulate the cytotoxic drug clodronate; these liposomes are used for the depletion of phagocytic cells as they are selectively taken up by phagocytes and cause cell death.

monocytes and the local proliferation of these cells within the injured kidney[74,75]. The degree of macrophage accumulation correlates with the severity of renal dysfunction, glomerular hypercellularity, segmental lesions and crescent formation in proliferative forms of glomerulonephritis[75,76] as well as renal allograft rejection in models of transplantation[77,78]. The macrophage infiltrate in rapidly progressive glomerulonephritis has a dominant M1 pro-inflammatory phenotype characterized by the expression of NOS2, TNF, IL-1β, CD80 and CD86

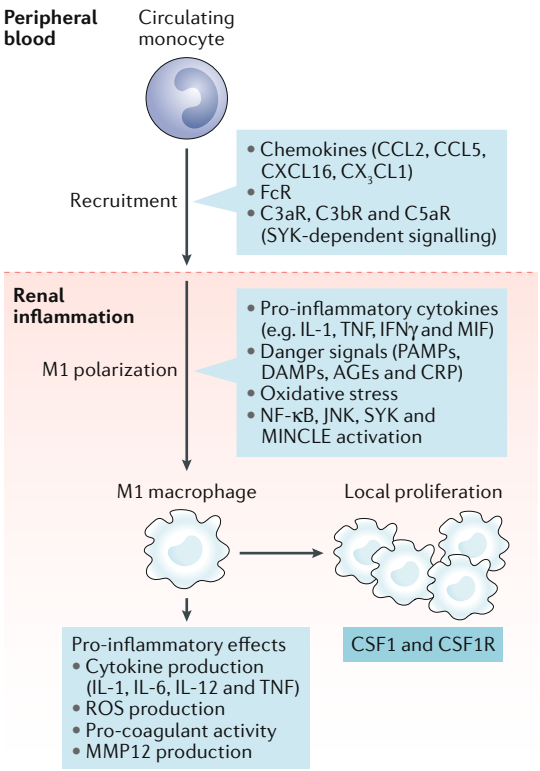

Fig. 2 | **Induction of M1 macrophages in renal inflammation.** The production of chemokines, deposition of immunoglobulins and complement activation recruit blood monocytes into the injured kidney, a process that is partly dependent on the spleen tyrosine kinase (SYK). Recruited monocytes can proliferate in the kidney in response to colony-stimulating factor 1 (CSF1), which amplifies the inflammatory response. The cytokines, oxidative stress and both endogenous and exogenous danger signals present in the inflammatory kidney microenvironment can trigger a variety of signalling pathways that promote macrophage polarization towards a pro-inflammatory M1 phenotype. These M1 macrophages are pro-coagulant and produce cytokines, oxygen radicals and proteolytic enzymes that not only increase the inflammatory response but can also cause renal injury. AGEs, advanced glycation end-products; CRP, C-reactive protein; CSF1R, CSF1 receptor; CX_3CL1, fractalkine; DAMPs, damage-associated molecular patterns; FcR, crystallizable fragment receptor; JNK, JUN N-terminal kinase; MIF, macrophage migration inhibitory factor; MINCLE, macrophage-inducible C-type lectin; MMP12, matrix metalloproteinase 12; NF-κB, nuclear factor-κB; PAMPs, pathogen-associated molecular patterns; ROS, reactive oxygen species; TNF, tumour necrosis factor.

(REFS[79–81]) (FIG. 2). The ability of macrophages to induce renal injury during inflammation has been demonstrated by studies that have used macrophage depletion techniques (such as the use of clodronate liposomes or the administration of diphtheria toxin to transgenic mice in which the diphtheria toxin receptor is specifically expressed on macrophages) in animal models of crescentic glomerulonephritis, acute renal allograft rejection and renal IRI[82–86]. However, a limitation of such techniques is that they deplete different macrophage populations. In the context of renal IRI, diphtheria-toxin-induced depletion of $CD11c^+$ cells (that is, dendritic cells and macrophages) resulted in a more complete depletion of $F4/80^+$ macrophages than the use of clodronate liposomes, which did not deplete the $CD206^+$ macrophage population. Notably, macrophage depletion with clodronate liposomes but not $CD11c^+$ cell depletion was protective against IRI-induced AKI, identifying a renoprotective population of $CD11c^+$ macrophages[84].

Monocyte proliferation and recruitment

Macrophages that accumulate in the injured kidney are almost entirely derived from bone marrow monocytes; the local proliferation of these macrophages is a feature of models of inflammatory renal injury[12,87]. Administration of a neutralizing CSF1R antibody that inhibits macrophage proliferation in the kidney without affecting blood monocyte levels demonstrated that CSF1-driven proliferation makes a substantial contribution to macrophage accumulation in models of renal allograft rejection, unilateral ureteric obstruction (UUO) and diabetic kidney disease[88–90]. Furthermore, inhibition of CSF1R signalling suppresses monocyte production in the bone marrow, which dramatically reduces the accumulation of kidney macrophages and provides considerable protection from renal injury in models of crescentic glomerulonephritis and acute renal allograft rejection[20,91].

In the inflamed kidney, CCL2 also mediates the recruitment and accumulation of blood monocytes[92], processes that are substantially reduced following CCL2 blockade in models of kidney injury, including diabetic kidney disease[93–96]. CCL2 blockade has a protective effect, and these studies demonstrate the importance of monocyte recruitment to the generation of M1 pro-inflammatory macrophages and establish such infiltrates as an important cause of kidney damage[97]. However, not all monocyte recruitment operates via CCL2. The chemokine fractalkine (CX_3CL1) facilitates the recruitment of blood monocytes into the injured kidney through its receptor, CX_3CR1; this process promotes renal injury following IRI and enhances renal fibrosis after UUO[98,99]. Whether targeting CX_3CR1 might be beneficial in kidney disease remains to be determined[100]. A number of other monocyte chemotactic molecules, such as CXCL16 (REF.[101]), CCL5 (REF.[93]), macrophage migration inhibitory factor (MIF)[102,103] and osteopontin[104], are also upregulated in human kidney disease and contribute to macrophage accumulation and renal damage in animal disease models. This array of chemotactic and growth factors that facilitate

monocyte recruitment and accumulation in the injured kidney thus provides multiple potential therapeutic targets for the inhibition of macrophage-mediated renal injury.

Monocyte recruitment into the injured kidney can also be driven by activation of the complement cascade within the kidney, which leads to deposition of the complement component C3b and local release of the potent chemotactic molecules C3a and C5a[105]; these chemoattractants recruit monocytes that express receptors for C3a, C3b and C5a into the injured kidney[105,106]. In addition, immunoglobulin deposition in the kidney can induce macrophage accumulation and activation via crystallizable fragment (Fc) receptors (FcRs)[105,107]. FcRs, C3bR and leukocyte integrins share a common intracellular signalling pathway that involves spleen tyrosine kinase (SYK)[108] (FIG. 2). Activation of SYK is evident in infiltrating macrophages and neutrophils in human kidney disease, and is particularly prominent in patients with rapidly progressive glomerulonephritis[109,110]. *Syk* deletion in myeloid cells and/or systemic administration of selective SYK inhibitors suppresses macrophage accumulation and activation, and reduces renal injury and damage in models of renal IRI[111], acute antibody-mediated renal allograft rejection[112], UUO-induced renal fibrosis[113] and crescentic glomerulonephritis[110,114].

M1 macrophages in renal injury

A range of diverse stimuli can induce an M1 pro-inflammatory macrophage phenotype within the injured kidney (FIG. 2), including cytokines that promote monocyte recruitment such as IL-1, TNF and MIF; systemic blockade of these cytokines is protective in models of renal inflammation[115–120]. As mentioned before, microbial molecules are also potent inducers of the M1 pro-inflammatory response, and PAMPs such as LPS and bacterial (CpG) DNA strongly induce a macrophage M1 response in vitro[121,122]. Administration of LPS or bacterial (CpG) DNA in models of kidney disease increases the macrophage M1 response and exacerbates renal injury[123–125]. The release of endogenous DAMPs can also induce an M1 pro-inflammatory macrophage phenotype and exacerbate renal injury in models of both acute and progressive kidney disease[126–135]. Examples of these DAMPs include DNA and high mobility group protein B1 (HMGB1) released by damaged and necrotic cells, as well as advanced glycation end-products (AGEs) and C-reactive protein (CRP). MINCLE is a transmembrane pattern recognition receptor that is expressed by M1 macrophages in response to activation of the TLR4–nuclear factor-κB (NF-κB) pathway by bacterial ligands such as LPS and glycolipids as well as endogenous lipid-based DAMPs; deletion of MINCLE inhibits M1 macrophage-mediated AKI[45].

Adoptive transfer of IFNγ-stimulated M1 macrophages induces more severe glomerular injury than adoptive transfer of unstimulated macrophages in models of acute anti-glomerular basement membrane (anti-GBM) disease[120]. In addition, adoptive transfer of LPS-stimulated M1 macrophages induces more severe renal injury in adriamycin nephrosis than the transfer of unstimulated macrophages[136]. Activation of NF-κB signalling is characteristic of M1 macrophages and promotes renal injury in rats with anti-GBM disease[137]. By contrast, macrophages in which NF-κB activation is inhibited acquire an anti-inflammatory phenotype and suppress renal injury following adoptive transfer[42]; these results emphasize the central role of NF-κB in M1 macrophage-mediated kidney damage.

JUN N-terminal kinase 1 (JNK) is also required for M1 polarization in vitro[121]; the adoptive transfer of macrophages in which JNK is inhibited[121] or the use of systemic JNK inhibitors[138,139] substantially reduced renal injury in a rat model of anti-GBM disease. These treatments did not, however, affect macrophage recruitment[140,141]. Other signalling pathways relevant to the polarization of M1 macrophages include the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathway, which is regulated by suppressor of cytokine signalling (SOCS) proteins. In fact, SOCS3 directs M1 polarization of monocyte-derived macrophages in rat models of peritonitis and nephrotoxic serum nephritis[142].

Aldosterone and miR-146 provide further examples of molecules that have a role in macrophage M1 polarization and that can regulate the extent of renal injury in experimental models. Aldosterone induces an M1 phenotype in macrophages[138,143] and conditional deletion of its receptor (that is, the mineralocorticoid receptor) in myeloid cells suppresses macrophage M1 polarization and reduces renal injury in mouse models of crescentic glomerulonephritis[144]. On the other hand, miR-146a suppresses the M1 pro-inflammatory response and diabetic mice deficient in miR-146a have an increased M1 response and a diminished M2 response, which exacerbates renal injury[145].

Macrophages induce renal injury through a diverse range of mechanisms, including the production of M1 macrophage effector molecules. Although macrophages are not major producers of IL-1β or TNF during renal injury[119,139], M1 macrophages can amplify the production of these cytokines during acute renal inflammation[20]. M1 macrophages stimulated with LPS and IL-1 produce MMP12 (REF.[141]); genetic or pharmacological blockade of MMP12 reduces macrophage infiltration and activation, thereby preventing crescent formation and severe glomerular injury[146,147], although the factors by which MMP12 mediates this effect have not been identified. Macrophage expression of tissue factor, the initiator of the primary coagulation pathway, is also upregulated by LPS and IFNγ[148]. Depletion of the predominant M1 macrophage infiltrate in a rat model of anti-GBM disease prevented fibrin deposition[20], suggesting that M1 macrophages might also promote renal injury through effects on coagulation.

Chronic inflammation and renal fibrosis

As discussed above, most forms of acute renal inflammation feature macrophage infiltration with a predominant M1 phenotype. These infiltrates transition towards an alternatively activated M2 phenotype in the chronic

phase of disease, although M1 macrophages remain a feature of chronic inflammation. The progression of chronic kidney disease (CKD) to ESRD is closely linked to the increased deposition of collagens and other extracellular matrix proteins, which accompanies the loss of normal kidney architecture[149,150]. In terms of renal pathology, fibrosis of the tubulointerstitial compartment is the strongest predictor of disease progression[151,152] and macrophage infiltration is a common feature of active fibrotic lesions. In fact, in human kidney diseases and chronic allograft injury, macrophage accumulation significantly correlates with both the degree of glomerulosclerosis and the extent of interstitial fibrosis and tubular atrophy[75,153–156].

M1-to-M2 transition in inflammation

The transition of macrophages from an M1 to an M2 phenotype is well documented in animal models of kidney disease but is not well defined in human kidney disease owing to the lack of repeat biopsies and the confounding effects of immunotherapies that directly affect macrophages. For example, glucocorticoids induce M2 macrophage polarization, characterized by the expression of CD163 and CD206 (REFS[157,158]).

Macrophage transition is best characterized in models of AKI, in which M1 cells are replaced by M2 cells during the repair response; available evidence suggests that the M2 cell population contributes extensively to recovery from injury[49]. For instance, numbers of NOS2-expressing M1 pro-inflammatory macrophages peak at day 2 post-reperfusion in mice with IRI, coinciding with the peak of injury, and are then replaced during the repair phase by M2 macrophages that express arginase 1 and CD206 (REF.[50]). Adoptive transfer of dye-labelled bone-marrow-derived macrophages that were stimulated with IFNγ demonstrated that M1 macrophages can switch to an M2 phenotype within the kidney during repair. In addition, adoptive transfer of IL-4-polarized M2 macrophages but not IFNγ-polarized M1 macrophages promoted renal tubular proliferation[50], which is indicative of renal repair. In fact, the presence of M2 macrophages seems to be a general feature of renal repair following AKI, as IL-4 and/or IL-13-mediated macrophage M2 polarization was also required for tubular recovery in a mouse model of AKI in which death of proximal tubular cells was induced by diphtheria toxin[52]. In addition, a switch of macrophage phenotype from M1 to M2 was observed in a mouse model of rhabdomyolysis-induced AKI[159], whereby F4/80^{low}CD11b^{high}Ly6b^{high}CD206^{low} M1 kidney macrophages were dominant 2 days after the induction of rhabdomyolysis, whereas F4/80^{high}CD11b^{+}Ly6b^{low}CD206^{high} M2 cells were dominant by day 8. Importantly, analysis at the single-cell level indicated the existence of considerable phenotypic heterogeneity, with some macrophages expressing both M1 and M2 markers[159]. An M1-to-M2 transition was also evident in a UUO model of renal fibrosis, which was promoted by the actions of tissue-type plasminogen activator[160].

A switch from an M1 to an M2 phenotype is also evident in models of glomerular disease. In a model of rat nephrotoxic serum nephritis, up until day 14, which is the peak of glomerular inflammation and cellular crescent formation, the macrophage infiltrate is predominantly of an M1 phenotype as the cells express high levels of NOS2 and MMP12. However, the progression of glomerular and interstitial fibrosis coincides with a reduction in macrophage numbers and a switch to an M2 phenotype, characterized by reduced expression of NOS2 and MMP12, and increased expression of CD163 and CD206 (REF.[161]).

M2 macrophages in renal fibrosis

The accumulation of M2 macrophages expressing CD206 and/or CD163 correlates with renal fibrosis in human kidney disease. In patients with diabetic kidney disease, the number of glomerular CD163^{+} macrophages correlates with glomerulosclerosis, interstitial fibrosis and tubular atrophy[156]. In adults with newly diagnosed immunoglobulin A (IgA) nephropathy, interstitial CD163^{+} and CD204^{+} macrophages are present in areas of active fibrosis and produce connective tissue growth factor; the degree of mesangial matrix expansion in biopsy samples from these patients correlates with the number of CD163^{+} macrophages[162]. A retrospective study of kidney allograft protocol biopsy samples found that CD163^{+} macrophages frequently localize to areas of interstitial fibrosis, characterized by the deposition of type I collagen and accumulation of myofibroblasts that express smooth muscle α-actin (αSMA). Furthermore, the number of CD163^{+} macrophages, but not the number of CD3^{+} T cells, at years 1 and 5 post-transplantation correlated with the degree of interstitial fibrosis at 5 and 10 years post-transplantation, respectively[158]. A different study of protocol biopsy samples collected 1 year after transplantation reported that numbers of CD206^{+} M2 macrophages correlated with subclinical inflammation, tubular injury and progression of fibrosis[163]. Increased numbers of CD206^{+} and CD163^{+} M2 macrophages were also found within the peritoneal effluent of patients on dialysis with peritonitis, and the production of CCL18 by M2 macrophages correlated with progressive ultrafiltration failure and peritoneal fibrosis[164]. These clinical findings point to a profibrotic role for a subset of M2 macrophages and underpin the need to define the role of macrophages in renal fibrosis.

Consistent with clinical studies, animal models of kidney disease have revealed the presence of a predominant M2 macrophage phenotype during the fibrotic phase of disease[157,161,165–167]. Severe IRI models AKI progression to CKD; in this model, depletion of the macrophage infiltrate, which is predominantly M2, protects against progressive interstitial collagen deposition. Moreover, adoptive transfer of M2c macrophages, but not M1 macrophages, reversed the beneficial effects of macrophage depletion on renal fibrosis[56]. Similarly, macrophage depletion from day 4 in a model of UUO significantly reduced renal fibrosis, whereas the adoptive transfer of M2 macrophages promoted the accumulation of αSMA^{+} cells[168]. The Wnt ligand Wnt3a enhances M2 macrophage polarization induced by IL-4 or TGFβ1; conditional deletion of *Wnt3a* in myeloid cells attenuated macrophage accumulation and M2

Box 1 | **Potential sources of myofibroblasts in renal fibrosis**

The origin of myofibroblasts in renal fibrosis is a controversial issue[186]. Several resident kidney cell types can give rise to myofibroblasts, including pericytes and perivascular fibroblasts[199], interstitial erythropoietin-producing cells[251–254], endothelial cells[252,255] and tubular epithelial cells[256–258]. In addition, renal myofibroblasts derived from bone marrow leukocytes have been described in numerous studies[135,138,140,142,143] — these cells are suggested to arise via macrophage-to-myofibroblast transition (MMT) within the kidney or as a discrete population of fibrocytes. Across different studies, differences in the underlying cause of renal fibrosis and the stage of disease are likely to affect the precise contribution of individual sources of myofibroblasts, and the clinical relevance of these findings will depend on how well the animal models replicate the disease process. One limitation of many of these studies is their reliance on cell phenotype and lineage markers rather than the functional measurement of fibrosis. Of note, *Col1a1* deletion in bone marrow leukocytes reduces renal fibrosis in two distinct models of kidney disease[198], which indicates that fibroblasts derived from bone marrow cells are important contributors to renal fibrosis.

polarization, and reduced renal fibrosis in the mouse UUO model[169]. M2 macrophages also promote glomerulosclerosis, as evidenced in a rat model of anti-GBM disease in which depletion via CSF1R inhibition of the predominant M2 macrophage infiltrate over days 14–35 significantly reduced both glomerulosclerosis and interstitial fibrosis[161]. Similar to studies in humans, findings from animal models of CKD suggest that the presence of M2 macrophages promotes fibrosis and disease progression.

The macrophage scavenger receptor CD36, generally considered to be a marker of M2 macrophages, regulates oxidative fibrogenic signalling and promotes renal fibrosis, although whether these effects are related to the phagocytosis of apoptotic cells remains unclear[170]. A number of molecules secreted by macrophages — including IL-1, MMP2, MMP9, MMP12, galectin 3 and platelet-derived growth factor — have also been implicated in promoting fibroblast proliferation and activation in renal fibrosis[171–178]. Furthermore, the protective effect of conditional deletion of the mineralocorticoid receptor in myeloid cells demonstrates that many of the beneficial effects of mineralocorticoid receptor antagonism, including reduced blood pressure and renal fibrosis, are due to direct effects on macrophage-mediated tissue injury[144,179,180].

Myeloid-derived suppressor cells

MDSCs might also have a role in renal fibrosis, as inhibition of Ly6G$^+$ MDSCs' function using all-*trans*-retinoic acid in a mouse model of adenine-induced CKD exacerbated renal fibrosis, suggesting that these cells act to suppress fibrosis[181]. In addition, clinical studies have shown that two types of monocytic MDSC populations exist in patients following renal transplantation: patient-derived CD14$^+$ monocytic MDSCs, which are capable of suppressing proliferation of CD4$^+$ T cells, and CD11b$^+$CD33$^+$HLA-DR$^-$ MDSCs, which can promote the expansion of regulatory T cells in vitro and in vivo[182]. However, the role of monocytic MDSCs in renal fibrosis requires further investigation given conflicting reports regarding the contribution of these cells to fibrosis of different organs. Similarly to findings in the model of adenine-induced renal fibrosis, inhibition of monocytic MDSCs with all-*trans*-retinoic acid exacerbated fibrosis in a model of liver injury[181]. However, monocytic CCR2$^+$ MDSCs produce TGFβ1 and promote silica-induced lung fibrosis via activation of lung fibroblasts[183]; CXCR2$^+$ MDSCs are also associated with severe pulmonary hypertension in clinical samples and disease models[184]. Understanding the heterogeneity of MDSC populations in different tissue microenvironments[183] remains a challenging question in the field that is complicated by the lack of reliable lineage markers for these different subsets.

Macrophage-to-myofibroblast transition

Myofibroblasts are a subset of activated fibroblasts that are characterized by the expression of αSMA and are the main cell type responsible for pathogenic collagen production during tissue fibrosis[185]. The origin of myofibroblasts in renal fibrosis is controversial, and several cellular sources have been identified — both within the kidney and bone marrow[186] (BOX 1). We suggest that macrophages derived from bone marrow cells can differentiate into αSMA$^+$ myofibroblasts within the injured kidney[17,27–29,158] in a process termed macrophage-to-myofibroblast transition (MMT). Regardless of the source of myofibroblasts, it is clear that local proliferation within the fibrosing kidney is an important mechanism by which myofibroblasts rapidly accumulate following renal injury[187–189].

Experimental evidence indicates that bone-marrow-derived fibroblasts make a substantial contribution to the development and progression of tissue scarring[190,191]. Studies in chimeric mice identified a significant subset of myofibroblasts within the fibrosing kidney that originated from the bone marrow[187,190,192–194]. A further study[187] showed that 35% of myofibroblasts in a UUO model of renal fibrosis were derived from the bone marrow. Non-chimeric approaches also identified monocyte-derived myofibroblasts in association with renal fibrosis, for example, in the cystic, fibrotic kidneys of *Crim1* hypomorphic mice[195].

The study of MMT in human disease relies on the detection of intermediate cells that uniquely co-express macrophage markers, such as CD68, and myofibroblast markers, such as αSMA. Two-colour immunostaining can be used to identify cells expressing both of these markers in tissue sections, although the precision of this analysis is limited owing to the close proximity of macrophages and fibroblasts in areas of active fibrosis. A biopsy study involving patients with a variety of native kidney diseases identified such intermediate CD68$^+$αSMA$^+$ cells in patients with active fibrotic lesions but they were not detected in samples showing acute inflammation without fibrosis or in samples with inactive fibrosis[29]. The number of CD68$^+$αSMA$^+$ cells correlated with total myofibroblast numbers in tissues with active fibrosis. In addition, the majority of CD68$^+$αSMA$^+$ cells co-expressed CD206, suggesting that macrophages that undergo MMT are predominantly M2 (REF.[29]). CD68$^+$αSMA$^+$ cells are also seen in biopsy samples from patients with active chronic renal allograft injury, but the numbers of these cells are much lower in biopsy samples from patients with acute or non-active chronic rejection[28]. Numbers of CD68$^+$αSMA$^+$

cells in active chronic renal allograft injury negatively correlate with allograft function and positively correlate with the total myofibroblast population[28]. These findings are supported by studies of gender mismatch between kidney allografts and transplant recipients in which allograft infiltration by recipient mesenchymal cells was identified in patients with chronic renal allograft rejection[196].

In line with findings from human biopsy studies, cells that co-express markers of macrophages (F4/80) and myofibroblasts (αSMA) have also been identified in mouse models of UUO and chronic renal allograft rejection[27–29]. Moreover, adoptive transfer of GFP$^+$F4/80$^+$ macrophages isolated from the bone marrow into irradiated UUO mice gave rise to GFP$^+$αSMA$^+$ myofibroblasts within the obstructed kidney and increased renal fibrosis[27]. In a follow-up study, mice that had undergone UUO received an intravenous injection of labelled CD11b$^+$ bone marrow macrophages, which were recovered from the obstructed kidney 5 days later. Gene expression analysis showed that the cells that migrated to the fibrosing kidney had acquired the phenotype of collagen-producing myofibroblasts[27]. To determine the origin of myofibroblasts in models of UUO and chronic allograft rejection, we performed lineage tracing using *LysM^{Cre}Rosa26-tdTomato* mice, which express the Tomato fluorescent protein exclusively in cells of the myeloid lineage. Use of confocal microscopy and flow cytometry showed that approximately half of the αSMA$^+$ myofibroblast population were Tomato$^+$F4/80$^+$ in addition to αSMA$^+$collagen$^+$, indicating that they were of myeloid origin. Many of these CD68$^+$αSMA$^+$ cells also expressed CD206, again suggesting that they had transitioned from an M2 macrophage[28,29]. The evidence for MMT in two contrasting models of renal fibrosis and in human biopsy studies suggests that this mechanism is of general importance in kidney disease. Furthermore, TGFβ1 stimulation of cultured F4/80$^+$ bone marrow macrophages induces an MMT response in which cells develop a long spindle morphology and express CD206, αSMA stress fibres and type I collagen[27]. However, not all myeloid lineage tracing studies in models of renal fibrosis have been able to identify macrophage-derived cells that produce type I collagen, suggesting that further work is needed in this area[197].

One limitation of studies that rely on marker expression and lineage tracing studies is that they focus on the phenotype of the cell rather than the function. This important issue was addressed in a 2018 study[198] that used mice in which *Col1a1* was specifically deleted in CD45$^+$ leukocytes to demonstrate that bone-marrow-derived leukocytes contribute 38–50% of type I collagen deposited in the kidney in models of UUO and adenine-induced nephropathy[198]. This functional study is important given that the contribution of bone marrow macrophages to the renal myofibroblast population in fibrosis is a contested issue[199] (BOX 1). One parabiosis study reported that in the UUO model, bone-marrow-derived myofibroblasts accounted only for ~10% of myofibroblasts in the kidney. This study used CD45$^+$ as the leukocyte marker and PDGFRβ$^+$ as the myofibroblast marker[200]; however, PDGFRβ$^+$ might not be expressed on all myofibroblasts and, because the timing of CD45 downregulation relative to the acquisition of a myofibroblast phenotype is not clearly defined in MMT studies, differences in the timing of analyses might also affect results in different studies. Whether macrophages that undergo MMT in the fibrosing kidney are derived from the general circulating monocyte pool or from a distinct subset of cells that are released from the bone marrow into the circulation in response to kidney damage remains to be determined. If these cells are from a distinct subset that is released from bone marrow, it might not be possible to detect them with a parabiosis approach in short-term models of renal fibrosis.

Another important issue relates to the difficulty in distinguishing bone-marrow-derived fibrocytes from MMT-derived myofibroblasts. It is proposed that an immature CD34$^+$CD11b$^+$CD45$^+$ population of myeloid precursor bone marrow cells differentiates into CD45$^+$type I collagen$^+$ fibrocytes, which can be found in the circulation and in the spleen[201–203]. Fibrocytes can promote angiogenesis and wound repair, but they may also contribute to collagen deposition in chronic fibrotic diseases[202]. However, whether fibrocytes maintain expression of macrophage lineage markers outside of the bone marrow compartment is uncertain, although one report describes CD11b expression by fibroblasts in the spleen and in the fibrosing kidney[203]. In addition, the lack of a specific fibrocyte marker makes it difficult to isolate this population for characterization and lineage tracing studies. However, the fibrocyte and MMT postulates share a common element in that they propose that bone marrow myeloid progenitors give rise to cells that infiltrate the kidney and contribute substantially to the myofibroblast population that promotes renal fibrosis.

***Role of* TGFβ1–*Smad3* signalling in MMT.** TGFβ1 has a crucial role in suppressing inflammation and promoting tissue repair, but, paradoxically, it can also promote chronic fibrotic diseases[149,204]. In progressive forms of human kidney disease, both the expression and activation of TGFβ1 are increased[205–208]. TGFβ1 is also a major inducer of M2 macrophage polarization[209–211] and promotes the differentiation and accumulation of myofibroblasts within the fibrosing kidney, as evidenced by a reduction in myofibroblast numbers following conditional deletion of *Tgfbr2* (encoding TGFβ receptor 2) in αSMA$^+$ cells[187]. However, TGFβ1 has many biological functions in addition to its role in fibrogenesis, which include the regulation of the T cell immune response[212,213]. Therefore, in order to identify potential therapeutic strategies to inhibit tissue fibrosis, it is important to determine which downstream signalling events separate the profibrotic actions from the regulatory functions of TGFβ1.

Canonical TGFβ signalling via TGFβR1 and TGFβR2 (also known as TGFR1 and TGFR2, respectively) operates via complexes that contain Smad2, Smad3 and Smad4 (REF.[149]) (FIG. 3). *Smad3*$^{-/-}$ mice have a profound reduction in myofibroblast accumulation

Fibrocytes
A population of immature myeloid cells in the bone marrow that differentiate into CD45$^+$type I collagen$^+$ fibroblast-like cells that are released into the circulation and can promote wound repair and tissue fibrosis.

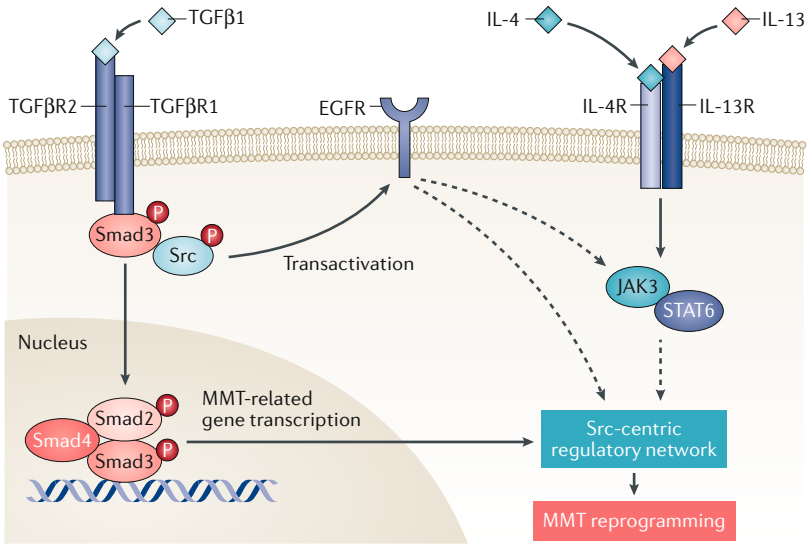

Fig. 3 | **Regulation of the macrophage-to-myofibroblast transition.** The macrophage-to-myofibroblast transition (MMT) process is driven by canonical transforming growth factor-β1 (TGFβ1)–Smad3 signalling in macrophages derived from bone marrow cells[27]. Binding of TGFβ1 to its receptor (that is, TGFβR1–TGFβR2) results in the formation of complexes that contain Smad2, Smad3 and Smad4 (REF.[149]); these Smad complexes then enter the nucleus and transcribe genes that are required for MMT[27,217]. Smad3 can also directly activate the tyrosine kinase Src, which induces fibroblast proliferation and activation[224–226]; in fact, the MMT process involves a Src-centric regulatory network[222]. Activation of the epidermal growth factor receptor (EGFR) also promotes fibroblast proliferation and renal fibrosis, and Src is involved in both TGFβ-induced transactivation of the EGFR and in EGFR-mediated activation of the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) signalling pathway[223,227]. In addition, IL-4 and IL-13 stimulation can induce MMT via JAK3–STAT6 activation[231,232]. Src might thus serve as a key checkpoint in several pathways of renal fibrosis, including MMT. IL-13R, IL-13 receptor; IL-4R, IL-4 receptor.

in the kidney and are protected against renal fibrosis across different disease models[214–216]. Adoptive transfer of GFP$^+$*Smad3*$^{-/-}$ or GFP$^+$*Smad3*$^{+/+}$ bone marrow macrophages into irradiated wild-type mice with UUO-induced kidney injury resulted in equal accumulation of GFP$^+$ macrophages within the obstructed kidney; however, only the GFP$^+$*Smad3*$^{+/+}$ macrophages transitioned to GFP$^+$αSMA$^+$ myofibroblasts and increased renal fibrosis. These findings establish a key role for Smad3 in the MMT process[27] and are consistent with an earlier study that reported that *Smad3*$^{-/-}$ mice have fewer bone-marrow-derived myofibroblasts and reduced fibrosis in response to UUO injury than do wild-type mice, and that deletion of *Smad3* from bone marrow protects against renal fibrosis[217]. In addition, transplantation of a renal allograft into a mismatched *Smad3*$^{-/-}$ recipient mouse resulted in dramatically lower numbers of CD206$^+$F4/80$^+$αSMA$^+$ MMT cells and reduced allograft fibrosis compared with transplantation into a wild-type mismatched recipient[28]. Notably, the duration of TGFβ1 exposure is an important factor in determining cellular phenotype in vitro. Short-term (2 days) exposure of bone-marrow-derived macrophages to TGFβ1 induces M2 polarization, but prolonged exposure for 5–7 days, representative of unresolved inflammation in kidney injury, promotes the transition of macrophages into collagen-producing αSMA$^+$ myofibroblasts[27,218–222] (FIG. 4).

Other mechanisms that regulate MMT. The regulatory gene networks involved in TGFβ1-induced MMT in F4/80$^+$*Smad3*$^{+/+}$ and F4/80$^+$*Smad3*$^{-/-}$ bone marrow macrophages have been examined at the transcriptional level using scRNA-seq. Bioinformatic analyses identified Src at the centre of a network of differentially expressed genes in TGFβ1-induced MMT[222]. Src is a widely expressed non-receptor protein tyrosine kinase that is activated by the triggering of various cell surface receptors, including immunoglobulin receptors, some receptors for PAMPs and DAMPs, integrins, focal adhesion kinases, receptor protein tyrosine kinases, G protein-coupled receptors and cytokine receptors[223]. Src participates in a wide range of signalling pathways, including the profibrotic epidermal growth factor receptor (EGFR) and JAK–STAT signalling pathways[223]. Src activation promotes fibroblast proliferation and activation in a variety of fibrotic diseases, including kidney disease[224–226]. In fact, Src inhibitors were highly effective in suppressing renal fibrosis in the UUO model — an effect that was associated with MMT inhibition[222,226]. In vitro studies show that Src is required for TGFβ1-induced MMT in bone-marrow-derived macrophages, and chromatin immunoprecipitation studies have demonstrated that TGFβ1 triggers Smad3 binding to the 3′ untranslated region of *Src* to increase its transcription[222]. In addition, Src is required for TGFβ1–Smad3-induced transactivation of EGFR in rat mesangial cells, which results in increased fibronectin production[227]. These findings identify Src as a key checkpoint in several major profibrotic signalling pathways (FIG. 3). Of note, a different member of the Src kinase family, haematopoietic cell kinase (also known as tyrosine-protein kinase HCK), has been identified in a profibrotic pathway in studies of chronic renal allograft injury; *Hck* overexpression led to activation of the TGFβ–Smad3 pathway in vitro. Furthermore, the non-selective kinase inhibitor dasatinib suppressed both HCK and Smad3 activation and reduced renal fibrosis in several models of kidney disease[228].

The T_H2 cytokines IL-4 and IL-13 can induce macrophage M2 polarization and promote fibrosis in various organ-based diseases through the activation of the JAK–STAT pathway[229]. In the kidney, the ability of CD4$^+$ T cells to promote renal fibrosis is associated with a T_H2 phenotype and the production of high levels of IL-4 and IL-13 (REF.[230]). Mice lacking the IL-4 receptor α-chain are protected from renal fibrosis induced by UUO and folic acid, concomitant with reduced STAT6 signalling in the kidney and reduced numbers of both CD206$^+$ M2 macrophages and CD206$^+$PDGFRβ$^+$ bone-marrow-derived fibroblasts[231]. In addition, IL-4 stimulation of cultured bone marrow monocytes induces STAT6 activation and promotes their transition to αSMA$^+$ myofibroblasts[231]. Consistent with these findings, the use of a JAK3 inhibitor in vitro prevented STAT6 activation and the MMT response in mouse monocytes stimulated with IL-4 or IL-13; in vivo, this inhibitor substantially reduced

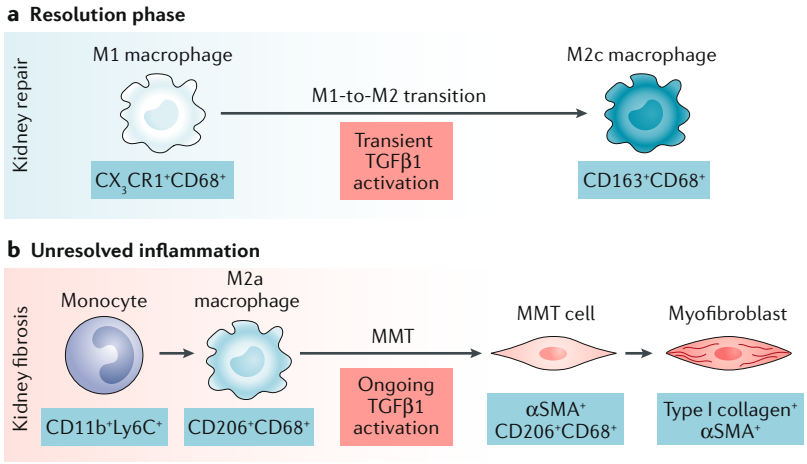

Fig. 4 | **Duration of TGFβ1 signalling determines macrophage phenotype in renal repair and fibrosis. a** | Following renal injury, if the underlying renal insult is resolved, short-term activation of transforming growth factor-β1 (TGFβ1) triggers macrophage polarization to an M2c phenotype, which induces immunosuppression, matrix remodelling and wound healing[9,52,57,59,60,182,260]. **b** | Unresolved inflammation increases the production and activation of TGFβ1 by infiltrating leukocytes and resident kidney cells. This ongoing TGFβ1 activation induces the transition of macrophages from an M2a phenotype (CD206$^+$) to myofibroblasts within the injured organ — a process termed macrophage-to-myofibroblast transition (MMT)[17,27–29,222,224]. Cells that co-express CD68 and smooth muscle α-actin (αSMA), which are macrophage and myofibroblast markers, respectively, represent an intermediate stage in the MMT process[27]. These MMT-derived myofibroblasts promote the development and progression of renal fibrosis.

the accumulation of CD11b$^+$PDGFRβ$^+$ bone-marrow-derived fibroblasts and renal fibrosis in the UUO model[232]. Furthermore, STAT6 deletion in the bone marrow compartment was sufficient to markedly reduce both the accumulation of CD11b$^+$PDGFRβ$^+$ bone-marrow-derived fibroblasts and renal fibrosis in the obstructed kidney[232].

Therapeutic prospects

Targeting macrophage-mediated renal injury is a difficult proposition. On the one hand, we need to inhibit the pro-inflammatory and profibrotic functions of macrophages that cause acute and progressive kidney disease (FIG. 5); on the other hand, it is important to preserve the ability of macrophages to contribute to tissue repair, defend against infection and regulate the immune response as MDSCs.

As mentioned previously, CCL2 is an important monocyte chemoattractant, and genetic and pharmacological blockade of CCL2 or its receptor CCR2 is protective in models of renal inflammation and fibrosis[64,67,103–105]. These findings led to a successful phase II trial of a CCR2 antagonist in diabetic kidney disease[233], which is now in phase III. Tubular cell production of CXCL16 promotes the recruitment of CXCR6-expressing monocytes and/or macrophages from the bone marrow, which results in accumulation of CD45$^+$type I collagen$^+$ cells and increased fibrosis in the UUO model[234,235]. Thus, targeting CXCL16 or its receptor CXCR6 might provide a more selective approach for preventing the accumulation of bone- marrow-derived myofibroblasts in CKD. CCL21 and its receptor CCR7 also facilitate accumulation of macrophages and CD45$^+$type I collagen$^+$ fibroblasts in the UUO model, which is linked to the expression of CCL21 in MECA79$^+$ lymphatic vessels and CCL21 production in secondary lymphoid tissues. Anti-CCL21 antibody treatment was effective in suppressing this infiltration of bone-marrow-derived fibroblasts and renal fibrosis[236], suggesting that it might have therapeutic potential.

Recruitment of monocytes into the injured kidney via integrins, FcRs, C3bR and C5aR operates via SYK signalling[17,110,111], which can be selectively inhibited to reduce macrophage recruitment and activation in models of renal inflammation and fibrosis[110,114]. In addition, the non-selective SYK inhibitor fostamatinib, which is also a potent JAK2 inhibitor, is highly effective in models of crescentic anti-GBM disease and lupus nephritis[237,238] and is now in a phase II trial in patients with IgA nephropathy[239].

Targeting CSF1R can reduce macrophage accumulation and renal injury without adversely affecting numbers of blood monocytes[88–90]. Small-molecule kinase inhibitors and neutralizing antibodies against CSF1R are being tested in clinical trials for the treatment of various types of cancer and aim to suppress the accumulation of M2 immunosuppressive tumour-associated macrophages[240]; CSF1R inhibitors have yet to be tested in patients with kidney disease.

JNK inhibitors can inhibit M1 polarization and are effective in models of renal IRI[241], UUO-induced fibrosis[242] and crescentic anti-GBM disease[140], even with delayed intervention[141]. A phase II trial of the pan-JNK inhibitor CC-930 in patients with idiopathic pulmonary fibrosis was halted early owing to acute liver toxicity, but markers of lung fibrosis were reduced[243]. Another JNK inhibitor (CC-90001), which preferentially inhibits JNK1 over JNK2, is now being assessed in a phase II trial in idiopathic pulmonary fibrosis. A successful outcome could pave the way for trials of JNK inhibition in acute and/or progressive kidney disease.

Src is another kinase implicated in renal fibrosis[223], and Src inhibitors were protective in animal models[222,224–226]. However, current Src inhibitors lack specificity and, whereas targeting several tyrosine kinases might be useful for the treatment of aggressive cancers[244], this lack of specificity is likely to be a major limitation in applying these treatments to patients with CKD.

Transcriptomic analyses of renal biopsy tissue identified a direct relationship between tubulointerstitial activation of the JAK–STAT pathway and the progression of renal failure in patients with type 2 diabetic nephropathy[245]. Although JAK–STAT signalling has important roles in macrophage activation, M2 polarization and MMT, this pathway is also critical for the T cell immune response and is activated in podocytes and tubular epithelial cells in kidney disease[246]. A phase II study of baricitinib, a JAK1 and JAK2 inhibitor, reduced albuminuria in patients with diabetic kidney disease[247], suggesting potential therapeutic benefit. Galectin 3 expression and secretion by macrophages promotes renal fibrosis via activation of renal fibroblasts in the UUO model[173], and serum galectin 3 levels correlate with progressive renal disease in type 2 diabetes[248]. A galectin 3 antagonist (GCS-100) is in phase II trials in diabetic kidney disease.

Tumour-associated macrophages
A subset of macrophages in the tumour microenvironment that inhibit immune cell-mediated destruction of tumour cells and promote tumour growth via stimulation of angiogenesis.

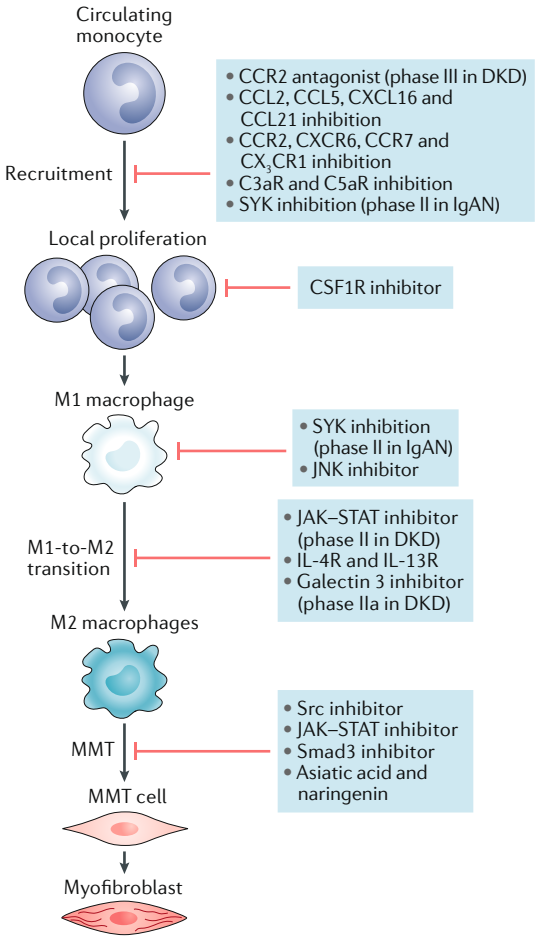

Fig. 5 | **Potential therapeutic targets in macrophage-mediated renal injury.** Monocytes can differentiate into M1 and M2 macrophages, and even myofibroblasts throughout the response to kidney injury; these differentiation programmes can be targeted to prevent renal damage. Targeting chemokines (such as CCL2, CCL5, CXCL16 and CCL21)[93,154,235,236], chemokine receptors (such as CCR2, CXCR6, CCR7 and CX_3CR1)[98,235,236,249,263,264], complement receptors (such as C3aR and C5aR) or spleen tyrosine kinase (SYK)[17,115,116,265] can prevent blood monocyte recruitment into the injured kidney. Macrophage proliferation within the kidney can be targeted with kinase inhibitors of colony-stimulating-factor 1 receptor (CSF1R)[266]. M1 polarization can be suppressed by inhibitors of JUN N-terminal kinase (JNK)[267] and SYK[113,265]. Macrophage M2 polarization induced by IL-4 and/or IL-13 can be targeted through cytokine receptor antagonism or inhibition of Janus kinase (JAK)–signal transducer and activator of transcription (STAT) signalling[52,268]. Galectin 3 is a macrophage product that increases myofibroblast differentiation and proliferation, and a galectin 3 inhibitor is currently being tested in clinical trials for the treatment of diabetic kidney disease (DKD)[68,173,269]. The transition of M2 macrophages into myofibroblasts can also be inhibited by blockade of Src[222] or JAK–STAT signalling[232], whereas the Smad3 inhibitor (SIS3)[28] and the Chinese herbs asiatic acid and naringenin[249] suppress the canonical transforming growth factor-β1 (TGFβ1)–Smad3 signalling that induces macrophage-to-myofibroblast transition (MMT). IgAN, IgA nephropathy; IL-13R, IL-13 receptor; IL-4R, IL-4 receptor.

Asiatic acid
A triterpenoid component extracted from *Centella asiatica*, which has anti-inflammatory and antifibrotic actions, including the increase in Smad7 expression.

Naringenin
A flavonoid from grapefruit and citrus fruits that has anti-inflammatory properties, including inhibition of Smad3 function.

An exciting area for future research is to target the MMT process itself as this seems to represent an essential checkpoint for the transition of inflammation to tissue fibrosis. The process of MMT emphasizes the importance of fine tuning TGFβ1–Smad3 signalling, as excessive signalling will drive MMT and renal fibrosis, whereas too little signalling could affect immune regulation. This balance has been achieved in studies that used Smad3 inhibitors, including the synthetic inhibitor SIS3 and active compounds derived from Chinese medicine (asiatic acid and naringenin), to 'recalibrate' TGFβ1–Smad3 signalling and inhibit renal fibrosis in disease models[187,222,249]. These strategies hold promise for future clinical studies.

Finally, scRNA-seq has the potential to comprehensively define cell types and states in human kidney biopsy specimens[250]. One analysis of 8,746 single-cell transcriptomes from a healthy adult kidney and a single kidney transplant biopsy core with mixed rejection enabled researchers to identify 16 distinct cell types, including all of the major immune cell types and most native kidney cell types. In this analysis, monocytes formed two subclusters — a nonclassical $CD16^+$ group and a classic $CD16^-$ group, which expressed dendritic cell maturation markers[250]. The application of scRNA-seq to human biopsy samples might therefore enable us to define major macrophage phenotypes across different disease states, define which macrophage populations predict disease progression versus resolution, identify candidate pathways by which macrophages cause renal damage, determine how macrophage phenotype is modified by immunosuppressive therapy and provide a detailed validation of how well experimental disease models translate to the clinic.

Conclusions

Macrophages can cause acute and chronic renal injury, but they also facilitate renal repair if the underlying insult resolves. These diverse functions, together with homeostatic roles in immune surveillance, are achieved through the remarkable plasticity of monocytes and macrophages, which can transition across a wide range of distinct functional populations that include M1 macrophages, different subtypes of M2 macrophages and MDSCs. Investigation of macrophage populations in human and experimental acute and progressive kidney disease has provided insights into the pathways that regulate macrophage function, including the identification of the MMT process in the pathogenesis of renal fibrosis. We propose that MMT represents an important checkpoint in the transition of renal inflammation to fibrosis during the progression to ESRD. Improved understanding of macrophage function is forthcoming with the advent of RNA sequencing of single cells isolated from human kidney biopsy samples. Current clinical trials targeting mechanisms involved in macrophage recruitment and activation, together with the multitude of preclinical studies, hold promise that we might be able to protect the kidney from macrophage-dependent inflammation and fibrosis.

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